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The goal of this DOD Breast Concept award was to identify and functionally characterize common genetic polymorphisms in the human UDP-glucuronosyltransferase gene, UGT1A9. We had previously determined that UGT1A9, a metabolic enzyme expressed predominantly in the human liver, catalyzed the glucuronidatic and inactivation of the antiestrogen raloxifene (RAL). The pharmacokinetics of RAL is known to be subject to significant interindividual variation, possibly associated with variable clinical efficacy. We hypothesized that genetic variation in the human UGT1A9 gene contributed to variable pharmacokinetics of RAL and therefore sought to characterize genetic polymorphisms within this gene. We describe here the identification of five common genetic polymorphisms within the human UGT1A9 gene. None of those polymorphisms altered encoded amino acid sequence. However, one variable length nucleotide repeat (VLNR) was within the putati` TATAA box promoter. The function of this polymorphism is currently under investigation.

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Rebecca B. Raftogianis, Ph.D.

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INTRODUCTION

The goal of this DOD Breast Concept award was to identify and functionally characterize common genetic polymorphisms in the human UDP-glucuronosyltransferase gene, UGT1A9. We had previously determined that UGT1A9, a metabolic enzyme expressed predominantly in the human liver, catalyzes the glucuronidation and inactivation of the antiestrogen raloxifene (RAL). The pharmacokinetics of RAL is known to be subject to significant interindividual variation, possibly associated with variable clinical efficacy. We hypothesized that genetic variation in the human UGT1A9 gene contributed to variable pharmacokinetics of RAL. The aims of this proposal were to 1) identify genetic polymorphisms within the coding regions of the human UGT1A9 gene, 2) functionally characterize recombinant UGT1A9 allozymes with regard to capacity to glucuronidate RAL and 3) express variant UGT1A9 cDNAs in MCF-7 cells and assess antiestrogenic response of cells to RAL.

BODY

Aim 1. Identify common genetic polymorphisms in the human UGT1A9 gene. The UGT1A9 gene is part of a nested UGT1A gene family on human chromosome 2. The organization of this locus is such that alternative transcription initiation occurs at promoters of eight unique first exons, followed by splicing to common exons 2 through 5. Thus, eight unique UGT1A isoforms are expressed from this locus and those proteins differ in sequence at the amino terminal 530 amino acids by virtue of the unique exon 1 and they each share identical carboxy-terminal protein sequence that is encoded by the common exons 2 through 5. We and others have previously shown a lack of variation in gene sequence within the shared exons 2 through 5. Thus, genetic variation in UGT1A genes lies predominantly in the unique first exon. Therefore, we initially characterized common genetic variation in the UGT1A9-specific first exon.

DNA was isolated from 65 healthy blood donors who donated blood samples to the Fox Chase Cancer Center Biosample Repository for the purposes of genetic research. This Repository operates with full IRB approval and all subjects have provided proper consent. DNA samples were provided to our laboratory in an anonymous fashion and no identifying information is available to us. This study also met with IRB approval (#01-832). The first exon as well as 5'UTR, 5' flanking and 3' intron sequences were amplified in 1 PCR reaction spanning 1466 nucleotides. All PCR reactions were subjected to automatic dye-terminator sequencing using a Perkin Elmer ABI 377 DNA sequencer in the Fox Chase Cancer Center DNA Sequencing Facility. Each PCR product was sequenced in the forward and reverse direction. Genetic polymorphisms were identified by comparing gene sequences with the computer program PolyPhred. This allows for identification of homozygously and heterozygously expressed polymorphic nucleotides. Determination of both sense and antisense sequence allowed for confirmation of polymorphic loci.

No genetic polymorphisms were identified within the coding region of UGT1A9. Genetic variants were identified within the 5'flanking region and 3' intron of UGT1A9. Table 1 describes those polymorphisms as well as their frequencies. Polymorphic loci were in genetic linkage such that different permutations of those polymorphisms defined eight apparent alleles. Of particular interest was the dT 9 or 10 variable length nucleotide repeat (VLNR) in the 5'flanking region of the gene. This position maps to the putative TATAA box of the UGT1A9 promoter. A TA repeat within the TATAA box of the human UGT1A1 protein has been extensively studied and shown to be functionally significant. The length of the repeat is inversely correlated with transcription and level of expression of the UGT1A1 protein. This polymorphism is associated with toxic response of individuals to the chemotherapeutic drug irinotecan.

Specific Aims 2 and 3. Functional Characterization of the UGT1A9 polymorphisms. Our original aims were to functionally characterize polymorphic UGT1A9 proteins (allozymes). However, none of the polymorphisms that we identified altered the encoded amino acid sequence of the protein. Therefore the experiments proposed would not be appropriate. It is possible that the VLNR in the putative promoter is functional. We are currently testing that hypothesis using standard luciferase reporter assays and constructs with either nine or ten "T" repeats at the polymorphic loci.

KEY RESEARCH ACCOMPLISHMENTS

● Identified five common genetic polymorphisms in the 5"flanking region and first intron of the human UGT1A9 gene.

● Determined the frequency and linkage of each of those polymorphisms in a population of 65 healthy Caucasian Americans.

● Currently evaluating the functional significance of the VLNR in the putative promoter.

Table 1. Genetic Variation in the Human UGT1A9 Gene

UGT1A9 Variable Loci	Nucleotide Polymorphism	Frequency
- 118 poly dT	T_9	0.56
	T_{10}	0.44
- 87	G	0.98
	A	0.02
I1 152	G	0.75
	A	0.25
I1 219	T	0.59
	A	0.41
I1 313	C	0.56
	A	0.44

-118 and -87 refer to nucleotide positions upstream of the "A" in the ATG
start codon. I1 refers to intron 1 and the number following the "I1" designation
refers to the nucleotide position downstream of the exon/intron junction.

REPORTABLE OUTCOMES

Jeffrey Zalatoris, Ph.D. and Rebecca Blanchard Raftogianis, Ph.D., UDP-glucuronosyltransferase-specific glucuronidation inactivates 4-hydroxytamoxifen and raloxifene. Abstract submitted to 2002 DOD Breast Cancer Era of Hope Meeting in Orlando, Fla.

CONCLUSIONS

We set out to characterize common genetic polymorphisms in the human UGT1A9 gene. Surprisingly, no polymorphisms affecting encoded amino acid sequence were identified. However, five common polymorphisms were identified in 5'flanking and intron regions of the gene. Of particular interest is the variable length nucleotide repeat in the putative promoter region of the gene. We are currently testing the hypothesis that this polymorphism is of functional consequence. Genetically determined variation in UGT1A9 activity may be an important factor in the clinical response of individuals to drugs that are metabolized via this pathway. This study has contributed toward our knowledge of common genetic variation in the human UGT1A9 gene.

REFERENCES

None

APPENDICES

None